

Claims

1. A polypeptide selected from muteins of the bilin-binding protein, characterized in that it

5 (a) is able to bind digoxigenin or digoxigenin conjugates,

(b) does not bind ouabain, testosterone and 4-aminofluorescein and

10 (c) has an amino acid substitution at at least one of the sequence positions 28, 31, 34, 35, 36, 37, 58, 60, 69, 88, 90, 95, 97, 114, 116, 125, and 127 of the bilin-binding protein.

2. The polypeptide according to claim 1, 15 characterized in that the dissociation constant of the complex with digoxigenin is 100 nM or less.

3. The polypeptide according to claim 1 or 2, characterized in that it carries, in comparison with 20 the bilin-binding protein, at least one of the amino acid substitutions selected from Glu(28)->Gln, Lys(31)->Ala, Asn(34)->Asp, Ser(35)->His, Val(36)->Ile, Glu(37)->Thr, Asn(58)->Arg, His(60)->Ser, Ile(69)->Ser, Leu(88)->Tyr, Tyr(90)->Ile, Lys(95)->Gln, Asn(97)->Gly, 25 Tyr(114)->Phe, Lys(116)->Ser, Gln(125)->Met, and Phe(127)->Leu.

4. The polypeptide according to claim 3, 30 characterized in that it has the amino acid sequence depicted as SEQ ID NO:15.

5. The polypeptide as claimed in one or more of claims 1 to 4, characterized in that it carries at 35 least one label group, selected from enzymatic label, radioactive label, fluorescent label, chromophoric label, (bio)luminescent label or label containing haptens, biotin, metal complexes, metals or colloidal gold.

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5 6. Fusion proteins of polypeptides according to one or more of claims 1 to 5, characterized in that an enzyme, another protein or a protein domain, a signal sequence and/or an affinity peptide is fused to the amino terminus of the polypeptide in an operable manner.

10 7. Fusion proteins of polypeptides according to one or more of claims 1 to 6, characterized in that an enzyme, another protein or a protein domain, a targeting sequence and/or an affinity peptide is fused to the carboxy terminus of the polypeptide in an operable manner.

15 8. A nucleic acid, characterized in that it comprises a sequence coding for a mutein or a fusion protein of a mutein of the bilin-binding protein according to one or more of claims 1 to 7.

20 9. The nucleic acid according to claim 8, characterized in that it comprises the nucleotide sequence according to SEQ ID NO:15 or another nucleotide sequence encoding the polypeptide according to SEQ ID NO:15.

10. A method for producing digoxigenin-binding muteins of the bilin-binding protein, which comprises the following steps:

30 (a) subjecting the bilin-binding protein to random mutagenesis at at least one of the sequence positions 28, 31, 34, 35, 36, 37, 58, 60, 69, 88, 90, 95, 97, 114, 116, 125, and 127,

35 (b) enriching resulting muteins with binding affinity for the digoxigenin group by selection and isolating said muteins,

(c) subjecting the muteins obtained in step (b) to another random mutagenesis at at least one of the sequence positions 28, 31, 34, 35, 36, and 37, and

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(d) again enriching the resulting muteins by selection and isolating said muteins.

11. The method according to claim 10, wherein in
5 step (b) selection is carried out by competitive enrichment.

12. The method according to claim 11, wherein
free digoxigenin or digitoxinigenin is used for
10 competitive enrichment.

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13. The method according to any of claims 10 to
12, wherein the enrichment in step (d) is carried out
by forming a complex of the muteins with the
15 digoxigenin group and subsequently dissociating the
complex.

14. The method according to claim 13, wherein the
dissociation of the complex of mutein and digoxigenin
20 group is carried out in acidic or basic medium milieu.

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15. A method for preparing a mutein or a fusion
protein of a mutein of the bilin-binding protein
according to one or more of claims 1 to 7 or for
25 preparing a mutein which is obtainable according to a
method according to one or more of claims 10 to 14,
characterized in that the nucleic acid coding for the
mutein or the fusion protein of a mutein of the bilin-
binding protein is expressed in a bacterial or
30 eukaryotic host cell and the polypeptide is obtained
from the cell or the culture supernatant.

15. The use of a mutein or a fusion protein of a
mutein of the bilin-binding protein according to one or
35 more of claims 1 to 7 or of a mutein which is
obtainable according to a method according to one or
more of claims 10 to 14 for binding, detecting,
determining, immobilizing or removing digoxigenin or
conjugates of digoxigenin with proteins, nucleic acids,

carbohydrates, other biological or synthetic macromolecules or low molecular weight chemical compounds.

- 5 17. A method for detecting the digoxigenin group,
wherein a mutein of the bilin-binding protein or a
fusion protein of a mutein of the bilin-binding protein
according to one or more of claims 1 to 7 or a mutein
which is obtainable according to a method according to
10 one or more of claims 10 to 14 is brought into contact
with digoxigenin or with conjugates of digoxigenin
under conditions suitable for effecting binding of the
mutein to the digoxigenin group, and the mutein or the
fusion protein of the mutein is determined.

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